IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: BARENHOLZ=8A

In re Application of:

Yeckezkel BARENHOLZ, et al.

Appln. No.: 10/073,365

Filed: February 13, 2002

For: CAROTENOID-LOADED LIPOSOMES

Art Unit: 1615

Examiner: G. KISHORE

Washington, D.C.

Confirmation No. 5480

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner for Patents
U.S. Patent and Trademark Office
Customer Service Window
Randolph Building, Mail Stop
401 Dulany Street
Alexandria, VA 22314

Sir:

I, Yeckezkel Barenholz, do hereby declare that a I am an inventor of the above-identified application.

In order to demonstrate that preparing liposomes by lyophilizing a suspension of a carotenoid and liposome-forming material produces unexpectedly superior liposomes to those prepared by conventional methods, including evaporating under nitrogen or drying in air, experiments were conducted under my direction and control.

Liposomes containing entrapped lycopene were prepared as follows:

a. Lycopene (2 mg) was dissolved in cyclohexane (4 ml), and weighted Egg-PC powder (E-PC) was added.

The E-PC:lycopene ration was E-PC(10):lycopene(1) (w/v). Two types of E-PC were used differing in their purity levels, Y E-PC and W E-PC (both from Lipoid).

b. The cyclohexane solvent was removed from the liposomes using one of the following drying methods:

(i) drying in air, at room temperature (RT);

(ii) evaporation under nitrogen, at RT; or

(iii) by lyophilization.

c. Hydration of the dried mixture of PC and lycopene was conducted with ultra pure water (18.2 megaOhem) resulting in the formation of six different liposome preparations.

For characterization, the liposomes were dissolved by x50 dilutions in isopropanol, providing clear solutions. The concentration of lycopene in each of the six preparations was measured by spectrophotometer at 472 nm.

The accompanying table and graph demonstrate that the drying conditions unexpectedly affect the concentration of intact lycopene in the liposomes, with lyophilization producing liposomes of higher concentration of entrapped lycopene than either air drying or drying under nitrogen.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the

like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 81 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon:

Yeckezkel Barenholz

20.02.05
Date

Lycopene entrapment in Egg-PC liposomes

Phospholipid	Method of drying (Temp.)	Dried form	Hydration	OD 472nm
-			-	0.0627
Y E-PC	lyophilization	Fluffy powder ("cake")	dispersed easily	1.2041
W E-PC	lyophilization	Fluffy powder ("cake")	dispersed easily	1.0042
W E-PC	N2 (RT)	film on the tube walls	poor hydration	0.4963
Y E-PC	N2 (RT)	film on the tube walls	poor hydration	0.7397
Y E-PC	air (RT)	adsorbed hard to the tube walls	not completely dispersable	0.2456
W E-PC	air (RT)	adsorbed hard to the tube walls	not completely dispersable	0.4372

Liposomes entrapped Lycopene were prepared as described in preparation II as follow:

Shortly, 2mg Lycopene dissolved in 4ml cyclohexane was added to weighted Egg-PC(**E-PC**) powder.

The PC :lycopene ratio was E-PC(10):Lycopen(1)(w/w) .

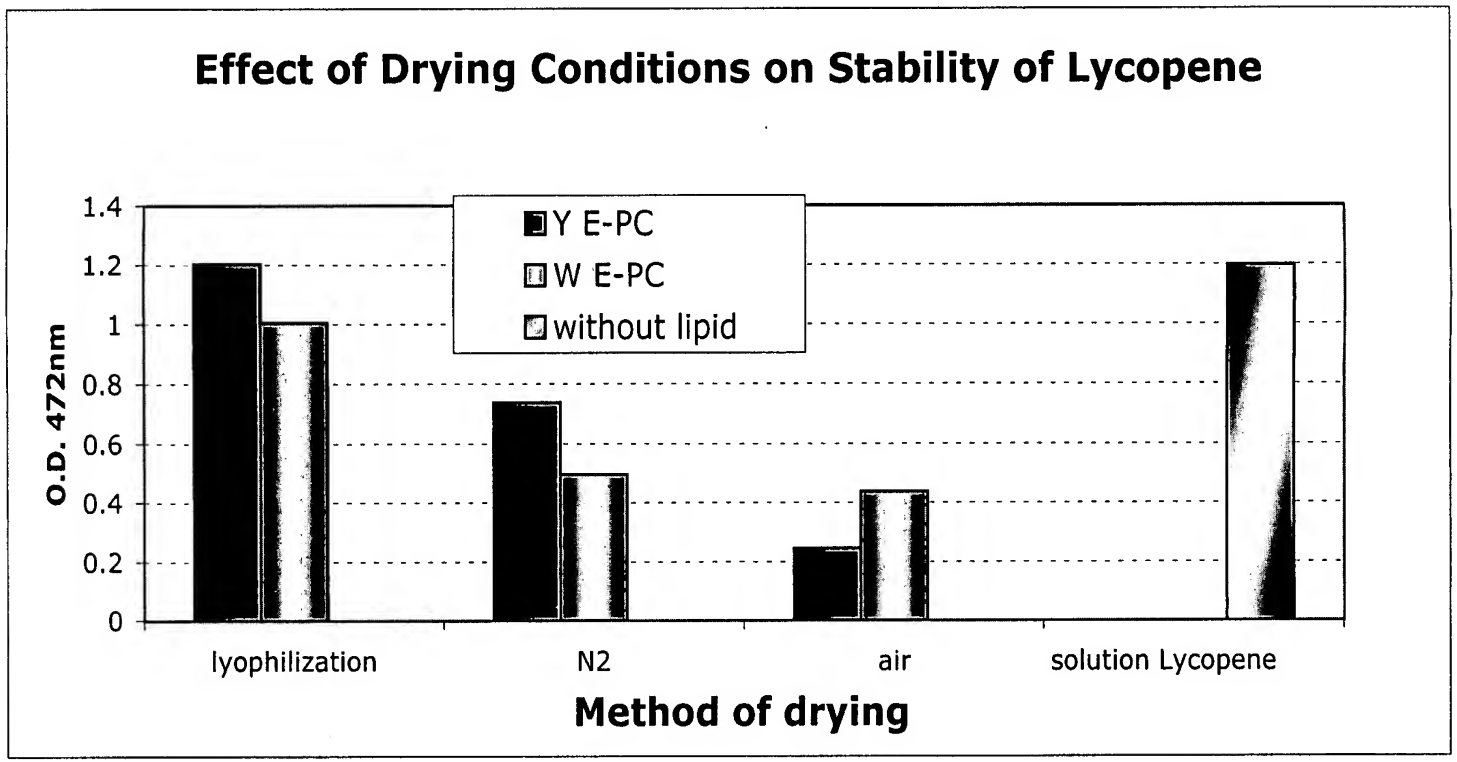
Two kind of E-PC (from Lipoid) differ in their purity levels , called **Y E-PC** and **W E-PC** were chosen.

The solvent was dried either using air or nitrogen at room temperature (RT), or lyophilized.

Hydration of the dried mixture of PC and Lycopene was done in ultra pure water (18.2 megaOhem).

The liposomes were diluted of x50 with isopropanol.The liposome solution became clear .

The concentration of lycopene in the liposome dispersion was measured by spectrophotometer- at 472nm.



Lycopene solution (control)- 2mg/ml Lycopene was dissolved in cyclohexane.

Conclusion: Lyophilization was the best way for drying mixture of phospholipids and Lycopene.